Isolation of Interphase Nuclei from Paraffin Section for FISH, Hedley Technique

Reagents

EDTA, 0.5 M
Ethanol, absolute
Formaldehyde, 37%
Nytal HD 55 filter (70 µm)
1X Phosphate Buffered Saline (PBS)
Proteinase K
EM Science, Gibbstown, Cat. 24568-
NaCl solution, 0.9% and 5 M
Tris-HCl, pH 7.5
Xylene
Water, sterile

Preparation

Proteinase K

Proteinase K	0.005 g
sterile H_2O	908 µl
Tris-HCl, pH 7.5	50 µl
0.5 M EDTA	20 µl
5 M NaCl.	2 µl

1% Formaldehyde/PBS

Formaldehyde	2.7 ml
1X PBS	97.3 ml

Procedure

1. Remove excess wax by incubating slides at 65°C for 2 hr.

2. Transfer slides immediately to jar containing xylene. Incubate at room temperature 2 x 10 min.

3. Wash slides 5 min each in 100%, 86%, and 70% ethanol.

4. Wash slides 2 x 2 min in 0.9% NaCl.

5. Transfer sections to a petri dish and mince the tissue by cutting with scissors.

6. Transfer to eppendorf tube containing proteinase K solution, incubate for 30 min at 37°C.

7. Filter suspension using a Nytal HD 55 filter (70 µm); wash the filter with 4 ml 1X PBS.

8. Centrifuge for 8 min at 1000 rpm.

9. Wash with 2 ml of 1X PBS, centrifuge for 8 min at 1000 rpm.

10. Resuspend pellet in about 20 µl of 1X PBS and drop onto clean glass slide or use a cytospin if possible.

11. Dry on a slide warmer at 37°C and then at room temperature overnight.

12. Rinse slides in PBS. Fix in 1% formaldehyde/PBS for 5 min, wash in PBS.

13. Drain excess fluid, dehydrate through ethanol series (70%, 90%, 100%), and air-dry.

14. Apply hybridization solution, add coverslip, and seal.

15. Denature the specimen at 85°C for 5 min and hybridize at 37°C for at least 16 hr. (Note: When probes are used that require a preannealing step, the denaturation of specimen and probe is performed separately.)